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(54) COLLAGEN SKIN DRESSINGS

(71) We, PIKOK INTERNATIONAL TRADING CO. LTD., a Hong Kong Company of Room 1006-8 American International Tower, 16 Queen's Road, Central, Hong Kong, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

This invention relates to skin dressings of collagen in sheet form and the production of such skin dressings. Throughout the specification the term "collagen" includes chemically modified collagen.

According to one aspect, the invention provides a process for the preparation of porous collagen skin dressing which comprises forming a collagen gel, extruding the collagen gel into a coagulation bath, forming a sheet of collagen from the extruded gel, cross-linking the extruded collagen, and subjecting the cross-linked sheet to freeze-drying. Preferably, the process comprises:

(a) treating a source of collagen with a proteolytic enzyme (other than collagenase) to form a telopeptide-poor collagen extract,

b) precipitating collagen from the extract,

c) purifying the precipitated collagen by redissolving and re-precipitation,

d) converting the extracted, purified collagen to a gel,

e) extruding the collagen gel through a tubular extrusion nozzle in a colagulation bath,

f) recovering collagen in tubular form from the coagulation bath,

g) slitting the tubular collagen longitudinally to form a collagen sheet therefrom,

h) cross-linking the longitudinal sheet, and

i) subjecting the cross-linked sheet to freeze-drying.

According to another aspect, the invention provides a process for the preparation of collagen skin dressing of limited porosity which comprises forming a collagen gel, extruding the collagen gel into a coagulating bath forming a sheet of collagen from the extruded gel, cross-linking the extruded collagen, partially air-drying the cross-linked sheet, and subjecting the partially air-dried sheet to freeze drying. Preferably, the process is used for the preparation of a collagen skin dressing in sheet form whose upper and lower surfaces possess differing porosity characteristics and comprises:

a) treating a source of collagen with a proteolytic enzyme (other than collagenase) to form a telopeptide-poor collagen extract,

b) precipitating collagen from the extract,

c) purifying the precipitated collagen by redissolving and reprecipitation,

d) converting the extracted, purified collagen to a gel,

e) extruding the collagen gel through a tubular extrusion nozzle in a coagulation bath,

f) recovering collagen in tubular form from the coagulation bath,

g) slitting the tubular collagen longitudinally to form a collagen sheet therefrom,

h) cross-linking the longitudinal sheet,

i) partially air-drying the cross-linked sheet, and

j) freeze-drying the partially air-dried sheet whereby the upper surface of said sheet becomes more concentrated in collagen content than the lower surface thereof.

Bactericidal agents or antibiotics may be impregnated into the sheet-type dressing.

A number of investigators including the present inventors have suggested the use of collagen material as a skin, burn or wound dresing. The feature of this invention, however, consists in the form of the collagen dressing and in the method of producing such desired types.

The U.S. National Fire Protection Association reported in 1962 that approximately

1,300,000 persons sustain burns yearly and occupy over 11,000 hospital beds per day. There is a great need for a readily available, easily stored and temporary substitute for human skin for the effective treatment of thermal burns and other forms of skin loss. It is common practice to cover skin loss area with split-thickness autografts, homografts and heterografts. Such treatments protect against infection, the loss of protein, fluid and electrolytes from exposed tissue. These treatments, however, have the following drawbacks. Grafts are difficult to obtain, and to store for any prolonged period of time and also are quite expensive. These difficulties could be reduced by the development of artificial skin dressings which are inexpensive and readily available to use.

Collagen is a major protein of connecting tissue such as skin, cornea, etc. and can be solubilized, separated and purified by the treatment with proteolytic enzymes (other than collagenase), e.g., proctase, pepsin, trypsin and pronase. Solubilized collagen is telopeptides-poor, relatively inexpensive and ideal as a material for development into a skin wound dressing.

Solubilized collagen has many NH_2 and COOH groups in its structure, and chemical modifications of the molecule can be readily made, e.g. all or some of the amino groups may be acylated by reaction with a mixture of acetic anhydride and acetic acid. Similarly, succinic anhydride reacts with collagen replacing amino groups by carboxyl groups. The carboxyl groups contained in the molecule are susceptible to esterification by the standard reaction with acidified alcohol, e.g., reaction with anhydrous methanol acidified with HCl. In the above reactions the net isoelectric point of collagen can be controlled, either negative or positive, or completely neutralized.

Various types of collagen and chemically modified collagen may be employed in the practice of this invention e.g. native, denatured collagen (neutral isoelectric point); esterified collagen (alkaline isoelectric point) and modified amino-group forms e.g. anhydride derivatives (acidic isoelectric point).

In a preferred preparation of the collagen gel, skin or hide is solubilized in an enzyme solution at acidic pH. The resulting gel is a viscous material which is recovered by filtering e.g. through cheese cloth and/or a millipore filter. The viscous solution is made alkaline by addition of caustic to a pH of about 10. At this stage the material is permitted to stand in order to inactivate any remaining enzyme. The material is thereafter neutralized, the collagen collected by centrifuge and washed with water. A second purification step follows, namely, redissolving in aqueous acid (pH 2.0 - 5.0), reprecipitation by neutralization to a pH of 6 to 7, and purification to remove acid by dialysis against water. The neutral gel is recovered and at this stage antibiotics or bactericides or both may be added before storage of the gel material.

Collagen material used in the preparation of gel is preferably not a multimer and, therefore, the material is not subjected to tanning during its preparation.

The porous sheet collagen skin dressing is preferably prepared as follows: Solubilized collagen gel (pH 2.0 - 3.5, collagen concentration 1% - 10%) is extruded from a tubular nozzle into coagulation bath (saturated NaCl). The coagulated tubular collagen is cut longitudinally to obtain sheet and tanned with 1 - 5% glutaraldehyde in saturated NaCl containing 0.05 M Na_2HPO_4 for 0.5 - 3.0 hours. The tanned collagen sheet is washed with water repeatedly, then freeze-dried on a methylmethacrylate plate. To produce a semi-porous, film type sheet in which the upper surface of the sheet is more concentrated in collagen (resulting in an upper film type surface) and in which the lower surface of the sheet is less concentrated in collagen (i.e. more porous) the sheet is subjected to partial air-drying prior to freeze-drying. The collagen sheet is sterilized with ethylene oxide gas and soaked in a typical base solution containing one or more bactericidal agents, such as silver nitrate (0.5 g/100ml), or silver lactate (0.5 g/100ml), or lactated Ringer's solution containing 25 mg/ml Gentamicin, 25 mg/ml Lincomycin, 25 mg/ml Colistimethate, 25 mg/ml Kanamycin, and 5 mg/ml Amphotericin B; or lactated Ringer's solution containing 25 mg/ml Lincomycin, 5 mg/ml Amphotericin B, and 25 mg/ml Gentamicin.

An effective skin dressing should have the following properties:

1. good adherence to the wound surface,
2. prevention of loss of protein, fluid and electrolytes,
3. prevention of infection,
4. reduction of pain
5. no stimulation of local tissue response, etc.

Collagen skin dressing as hereinafter described in detail have been found to satisfy the above properties and to be easy to use and relatively inexpensive. In particular, the porous sheet, and semi-porous sheet type dressing adhere firmly to the wound and give effective protection against infection and good wound healing.

The present invention may be further understood from the following examples:

Example 1

Fresh calfskin (about 5 kg) was dehaired, cleaned by shaving and cut into small pieces. The skin was washed repeatedly with 10%

NaCl containing a 0.2% sodium azide bactericide and with sterilized water. The skin was solubilized in 10 liters of water (pH 2.5 HCl) containing 30 mg/ml Gentamicin by addition of 1 g. of pepsin (approximate ratio of enzyme to collagen was 1/400) at 20°C for 4 days with intermittent stirring. The resulting viscous solubilized collagen was filtered through cheesecloth, its pH raised to 10 by NaOh and allowed to stand for 24 hours at 20°C to inactivate the pepsin. The pH of the collagen was then adjusted to 7-8 (HCl) and a collagen precipitate was collected by centrifuging and washed with sterilized water. The washed precipitate was redissolved in acidic solution and reprecipitated at pH 7-8 for further purification.

The collagen was dissolved in dilute HCl solution (final pH 2.5, collagen concentration was 3%) and deairated under vacuum. The collagen acidic gel was extruded into a coagulation bath (saturated NaCl) through an appropriate nozzle. Coagulated tubing was recovered and cut longitudinaly to make it into sheets and tanned with 3% glutaraldehyde in saturated NaCl containing 0.05 M Na_2HPO_4 for one hour. After repeated washing with water, the collagen sheet was freeze dried on a plate of methylmethacrylate. Freeze-dried sheets (10cm × 10cm) were sterilized by treatment with ethylene oxide gas and preserved by soaking in 0.5% silver nitrate solution. The final thickness of the sheet was 3 mm. This skin dressing had good adhesion to a wound, protection against fluid loss and infection, and wound healing.

Example 2

Collagen sheet was prepared by extrusion, tanning and washing by the method described in Example 1 but using 5% acidic collagen gel. The washed collagen sheet was then partially air-dried on a plate of methylmethacrylate until the thickness of the sheet became half of the original. This partial drying reduces the porosity (collagen concentration higher) of the upper surface of the sheet. It was then freeze-dried to render the lower surface porous (collagen concentration lower) and sterilized wih ethylene oxide gas. The sterilized sheet was preserved in sterile 0.5% silver lactate solution at pH 7.4) The final thickness of the sheet was 2 mm. This skin dressing had finer porosity and greater strength than the sheet of Example 1. It showed good protection against protein, fluid and electrolytes loss, protection against infection and wound healing.

Example 3

Sterile, freeze-dried collagen sheet was prepared by the method described in Example 1, except that the collagen concentration was 5%. The sheet was preserved by soaking in sterile lactated Ringer's solution (pH adjusted to 7.4) containing the following antibiotics: 25 mg/ml Gentamicin, 25 mg/ml Lincomycin, 25 mg/ml Colistimethate, 25 mg/ml Kanamycin and 5 mg/ml Amphotericin B. The final thickness of the sheet was 4 mm. This sheet likewise displayed good skin dressing properties.

WHAT WE CLAIM IS:

1. A process for the preparation of porous collagen skin dressing which comprises forming a collagen gel, extruding the collagen gel into a coagulation bath, forming a sheet of collagen from the extruded gel, cross-linking the extruded collagen, and subjecting the cross-linked sheet to freeze-drying.

2. The process of claim 1, which comprises

a) treating a source of collagen with a proteolytic enzyme (other than collagenase) to form a telopeptide-poor collagen extract,

b) precipitating collagen from the extract,

c) purifying the precipitated collagen by redissolving and re-precipitation,

d) converting the extracted, purified collagen to a gel,

e) extruding the collagen gel through a tubular extrusion nozzle in a coagulation bath,

f) recovering collagen in tubular form from the coagulation bath,

g) slitting the tubular collagen longitudinally to form a collagen sheet therefrom,

h) cross-linking the longitudinal sheet, and

i) subjecting the cross-linked sheet to freeze-drying.

3. The process of claim 1 or 2 in which the cross-linking is carried out by tanning with glutaraldehyde.

4. Collagen skin dressing prepared by the process of claim 1, 2 or 3.

5. Collagen skin dressing prepared by the process of claim 1, 2 or 3 containing at least one material selected from antibiotics and bactericidal agents.

6. A process for the preparation of collagen skin dressing of limited porosity which comprises forming a collagen gel, extruding the collagen gel into a coagulation bath, forming a sheet of collagen from the extruded gel, cross-linking the extruded collagen, partially air-drying the cross-linked sheet, and subjecting the partially air-dried sheet to freeze-drying.

7. The process of claim 6 for the preparation of a collagen skin dressing in sheet form whose upper and lower surfaces possess differing porosity characteristics which comprises:

a) treating a source of collagen with a proteolytic enzyme (other than collagenase) to form a telopeptide-poor collagen extract,

b) precipitating collagen from the extract,

c) purifying the precipitated collagen by redissolving and reprecipitation,

d) converting the extracted, purified collagen to a gel,

e) extruding the collagen gel through a tubular extrusion nozzle in a coagulation bath,

f) recovering collagen in tubular form from the coagulation bath,

g) slitting the tubular collagen longitudinally to form a collagen sheet therefrom,

h) cross-linking the longitudinal sheet,

i) partially air-drying the cross-linked sheet, and

j) freeze-drying the partially air-dried sheet whereby the upper surface of said sheet becomes more concentrated in collagen content than the lower surface thereof.

8. The process of claim 6 or 7 in which the cross-linking is carried out by tanning with glutaraldehyde.

9. Collagen skin dressing prepared by the process of claim 6, 7 or 8.

10. Collagen skin dressing prepared by the process of claim 6, 7 or 8 containing at least one material selected from antibiotics and bactericidal agents.

11. A process according to claim 1 or 6 substantially as hereinbefore described in any of the Examples.

12. A skin dressing according to claim 4 or 9 substantially as hereinbefore described in any of Examples.

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